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Short communication

Separation and identification of perchlorinated polycyclic aromatic hydrocarbons by high-performance liquid chromatography and ultraviolet absorption spectroscopy

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Abstract

High-performance liquid chromatography (HPLC) was coupled with ultraviolet absorption spectroscopy (UV) for the simultaneous separation and identification of a series of perchlorinated polycyclic aromatic hydrocarbons, such as perchlorobenzene (C_6Cl_6), perchloronaphthalene ($C_{10}Cl_8$), perchlorobiphenyl ($C_{12}Cl_{10}$), perchloroanthracene ($C_{14}Cl_{10}$), perchlorophenanthrene ($C_{14}Cl_{10}$), perchloroacenaphthylene ($C_{12}Cl_8$), perchloropyrene ($C_{16}Cl_{10}$) and perchlorofluoranthene ($C_{16}Cl_{10}$). HPLC was performed on an ODS column using methanol–hexane (80:20) as mobile phase at a flow-rate of 1.0 ml/min. UV absorption spectra of the elutes were detected in the region of 210–350 nm. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Perchlorinated polycyclic aromatic hydrocarbons (PAHs) is a family of compounds which have not been well studied. Interest in these compounds is partly due to their importance in environmental samples [1–3]. Methods of HPLC (high-performance liquid chromatography) have been developed to detect some chlorinated PAHs in chlorinated tap and surface water [4], in emissions from municipal waste incinerators [5], in urban air and in automobile exhausts [6], etc. However, HPLC separation of the

fully chlorinated PAHs has rarely been described in the chemical separation literature and the effort to develop chromatographic methods to separate the compounds will therefore merit attention in the related fields.

Recently, we have synthesized a series of perchlorinated PAHs by means of discharge in liquid or vapor chloroform [7,8], and recorded UV adsorption spectra of eight products, perchlorobenzene (**I**), perchloronaphthalene (**II**), perchlorobiphenyl (**III**), perchloroanthracene (**IV**), perchlorophenanthrene (**V**), perchloroacenaphthylene (**VI**), perchloropyrene (**VII**) and perchlorofluoranthene (**VIII**). Based on these standard perchlorinated PAHs, the technique of separating and determining these compounds was developed on a conventional ODS column by coupling HPLC with UV absorption spectroscopy.

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2. Experimental

2.1. Chemicals

Solvents used in the experiments were commercially available. Methanol was HPLC grade, and other solvents were analytical grade. All HPLC solvents were further distilled and degassed by vacuum filtration over a 0.45- μ m membrane filter prior to use.

2.2. Standard compounds

As most of the perchlorinated PAHs were not commercially available, all standard compounds for the experiments were prepared in the laboratory: **I–V** were synthesized by arc discharge in liquid chloroform [7], and **VI–VIII** were obtained from glow discharge in chloroform vapor [8]. Among them, **I** was separated and purified by sublimating the crude products at 160°C, and was characterized by MS and infrared spectroscopy (IR) [7]. Other standard compounds were separated on an open column using alumina as stationary phase, and were further purified by recrystallization. **IV** was identified by MS and ^{13}C nuclear magnetic resonance (NMR), and **II**, **III**, **V–VIII** were characterized by X-ray diffraction of single crystals [9–12]. UV adsorption spectrum of each standard compound was recorded for reference.

2.3. HPLC

The HPLC apparatus consisted of a TSP P200 pump and a TSP UV3000 detector. Data are processed by PC 1000 software. Separation was carried out at room temperature on a Spherisorb C_{18} column, 10 μ m particle size, 8 nm pore size, 200 \times 4.5 mm I.D. Chromatography was performed using a mobile phase of methanol–hexane (80:20). The flow-rate of the mobile phase was 1.0 ml/min. Sample injection was quantified with a 20- μ l loop. Peaks were detected over the 210–350 nm range of the absorption spectrum. The scan resolution and the data rate of the spectrometer were fixed at 5 nm and 6.7 Hz, respectively.

Calibration curves for the perchlorinated PAHs were constructed by plotting the peak area of each calibration standard at maximum absorption wave-

length as a function of its concentration. The detection limit was defined as the concentration which produced an absorption signal triplicate the peak-to-peak noise.

3. Results and discussion

Non-aqueous reversed-phase HPLC was selected considering the low volatility and high lipophilicity of these perchlorinated PAHs. In order to record UV spectra of the target compounds, the solvents need to be transparent or low absorption in the wavelength region of 210–350 nm. Thus, only a few solvents, such as methanol, ethanol, acetonitrile, tetrahydrofuran, hexane and cyclohexane, could satisfy the requirements. In the primary experiment, these solvents were individually applied as the mobile phase. However, while **VI–VIII** compounds could not be eluted from an ODS column when a polar solvent was applied, the selection could not be utilized in low-polar solvents due to the lack of retention. Hence, the mixture consisting a polar and a low-polar solvent was considered as mobile phase at the further experiment. After testing various combinations of the mixtures, it was found that solvents of some mixtures were not dissolved, while some mixtures, such as ethanol–hexane, acetonitrile–tetrahydrofuran and cyclohexane–ethanol, lacked retention, and only methanol–hexane could be used as mobile phase for separating the eight perchlorinated PAHs. Optimum conditions were sought by varying the ratio of methanol to hexane. The capacity factor (k') decreases and the solubility increases markedly as the concentration of hexane rises. After the tests, optimum ratio of methanol to hexane chosen for separating the compounds was 4:1.

Fig. 1 displays a HPLC run of the eight standard perchlorinated PAHs at 250 nm. As shown in the chromatogram, the peaks of the compounds were well separated with the exception of **VI** and **VII**. Retention times of these compounds obtained from five measurements are listed in Table 1. It is interesting to note from the data that eluting sequence of the perchlorinated PAHs can correlate with their structures, and their retention times rise with increase of the number of fused aromatic rings. The correlation has also been observed in the chromatog-

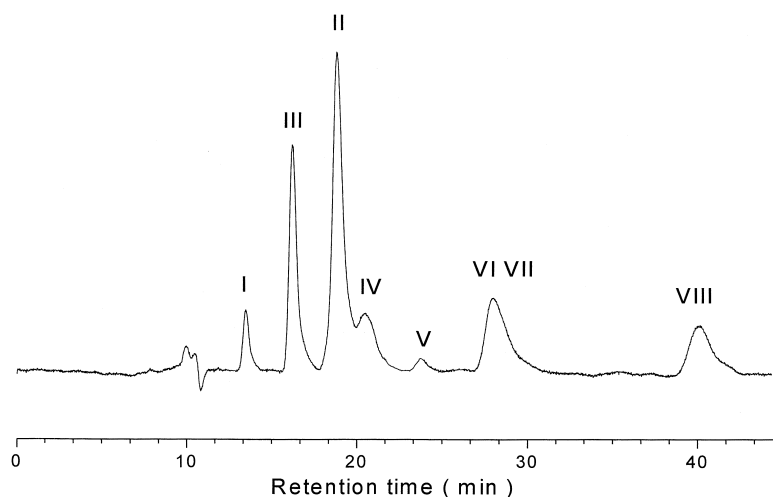


Fig. 1. Chromatogram of standard perchlorinated PAHs. Concentrations of perchlorobenzene (I), perchloronaphthalene (II), perchlorobiphenyl (III), perchloroanthracene (IV), perchlorophenanthrene (V), perchloroacenaphthylene (VI), perchloropyrene (VII) and perchlorofluoranthene (VIII) are 1.70, 2.09, 3.26, 1.52, 0.26, 1.09, 1.09 and 0.70 mg/l, respectively.

Table 1
Structures and analysis results of the perchlorinated PAHs

Compound	Formula	Structure	λ_{\max} (nm)	Correlation coefficient	DL ^a (ng/ml)	t_R^b (min)	k'^c
Perchlorobenzene (I)	C ₆ Cl ₆		215	0.9999	9.7	13.49	0.496
Perchlorobiphenyl (III)	C ₁₂ Cl ₁₀		215	0.9997	9.5	16.25	0.802
Perchloronaphthalene (II)	C ₁₀ Cl ₈		275	0.9992	1.5	18.87	1.092
Perchloroanthracene (IV)	C ₁₄ Cl ₁₀		265	0.9992	1.3	20.41	1.263
Perchlorophenanthrene (V)	C ₁₄ Cl ₁₀		295	0.9998	3.8	23.75	1.633
Perchloroacenaphthylene (VI)	C ₁₂ Cl ₈		265	0.9997	0.5	27.98	2.102
Perchloropyrene (VII)	C ₁₆ Cl ₁₀		215	0.9992	12.1	28.68	2.180
Perchlorofluoranthene (VIII)	C ₁₆ Cl ₁₀		275	0.9993	0.2	40.20	3.457

^a Detection limit.

^b Mean retention time of five measurements.

^c The t_0 was measured as retention time of methanol with hexane as mobile phase on the same stationary phase used in the HPLC analysis. t_0 = 9.02 min.

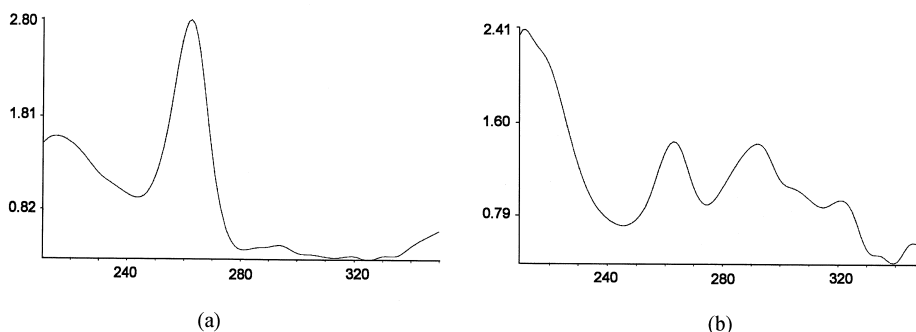


Fig. 2. UV spectra of (a) perchloroacenaphthylene (VI) and (b) perchloropyrene (VII).

raphy of their PAHs parents, but retention of the perchlorinated compounds are much stronger due to the chloro-substitution.

By coupling with the UV adsorption spectroscopy, the components of the sample were better resolved and identified. For instance, if the elutes were detected at single wavelength as the chromatogram shown in Fig. 1, perchloropyrene and perchloroacenaphthylene were not resolved, because their resolution was only 0.4 under this HPLC condition. As shown in Fig. 2, however, their UV spectra, which were acquired at their retention times respec-

tively, were markedly different, so the two compounds could still be determined by HPLC–UV.

Besides, detection limits of the perchlorinated compounds at maximum absorption wavelength are reported in Table 1. The table also lists correlation factors of these standard compounds, based on the peak area of the linear calibration graphs.

In addition to the characterization of the standard compounds, the products synthesized from discharge of liquid chloroform was also analyzed as an additional application by recording chromatogram coupled with their UV spectra. The sample was prepared

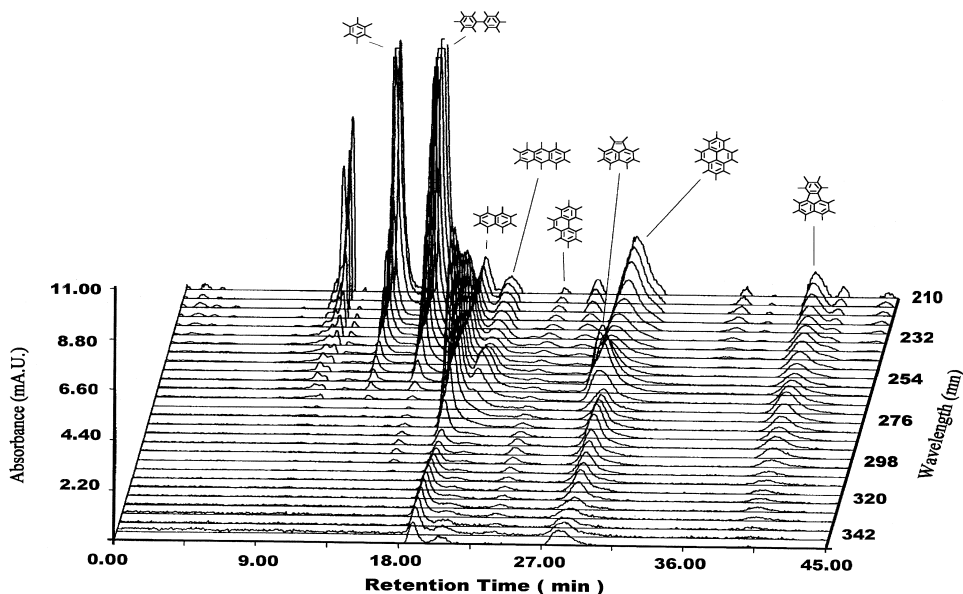


Fig. 3. Chromatogram of the products from discharge reaction of chloroform.

as following: crude products were sublimated at 160°C to exclude most of the volatile species, such as perchlorobenzene which was the major product of the discharge reaction and might overload the ODS column. The residues, which still contains little amount of perchlorobenzene, were extracted by toluene, followed by elution through a short open column using silica as stationary phase and petroleum ether as mobile phase. The eluates were dissolved in methanol–hexane (4:1), and filtered through a 0.45- μ m pore diameter filter prior to HPLC injection. Fig. 3 displays the three-dimensional chromatogram of the sample. From the chromatogram, the eight perchlorinated PAHs can be identified by both their retention times and UV spectra. In addition to the eight compounds, several unidentified components of the sample are also observed, but they still remain to be characterized.

Acknowledgements

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References

- [1] A. Colmsjö, A. Rannug, U. Rannug, *Mutat. Res.* 135 (1984) 21.
- [2] G. Löfroth, L. Nilsson, E. Agurell, T. Sugiyama, *Mutat. Res.* 155 (1985) 91.
- [3] R. Toftgård, B. Franzen, J.-Å. Gustafsson, *Environ. Int.* 11 (1985) 369.
- [4] J. Lehotay, K. Hromulakova, J. Liquid Chromatogr. Rel. Technol. 20 (1997) 3193.
- [5] H.P. Nirmaier, E. Fischer, A. Meyer, G. Henze, J. Chromatogr. A 730 (1996) 169.
- [6] P. Haglund, T. Alsberg, Å. Bergman, B. Jansson, *Chemosphere* 16 (1987) 2441.
- [7] R.B. Huang, W.J. Huang, Y.H. Wang, Z.C. Tang, L.S. Zheng, *J. Am. Chem. Soc.* 119 (1997) 5954.
- [8] S.Y. Xie, R.B. Huang, L.H. Chen, W.J. Huang, L.S. Zheng, *J. Chem. Soc., Chem. Commun.* 18 (1998) 2054.
- [9] G. Gafner, F.H. Herbstein, *Nature* 200 (1963) 130.
- [10] F.P. Berit, *Acta Cryst.* B31 (1975) 2931.
- [11] A.C. Hazell, S. Jagner, *Acta Cryst.* B32 (1976) 682.
- [12] F.H. Herbstein, M. Kapon, R. Merksamer, *Acta Cryst.* B32 (1976) 2205.